

Preliminary Amendment

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For: DRUG DISCOVERY METHODS

Please replace the paragraph beginning at page 29, line 18, with the following rewritten paragraph. Per 37 C.F.R. § 1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The adaptein library-containing vector DNA is isolated from *E. coli* bacteria by standard techniques and purified by equilibrium density gradient separation using cesium chloride to form the gradient and following standard techniques. The DNA (adaptein library) is dissolved at 1 mg/ml in 1mM EDTA, 10 mM Tris-HCl, pH 8.0 (TE) and stored at -80°C until required. To produce the MLV encoding the adaptein library, HEK 293 human fibroblasts are grown to 80% confluence on plastic plates in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (culture medium) and with the bacterial antibiotics streptomycin and penicillin. The cells are simultaneously transfected with vector DNA encoding the adaptein library (25 µg), the MLV structural genes gag-pol (25 µg) and the Vesicular Stomatitis virus glycoprotein (VSV-G; 5 µg). Transfection is conducted according to the methods of Chen and Okayama (*Mol. Cell. Biol.*, 7, 2745-2752 (1987) and used calcium phosphate to form a precipitate with the DNA that is efficiently taken up by this cell type and the encoded genes expressed. The DNA is mixed with 0.1 mM EDTA, 1 mM Tris-HCl, pH 8.0 in purified water to give a total volume of 1.25 ml. To this 1.25 ml of 50 mM BES, 280 mM NaCl, 1.5 mM Na₂HPO₄, pH 7.0 is added and mixed. Calcium chloride (2 M, 0.16 ml) is added dropwise while mixing and then incubated for 10 minutes at room temperature. This is then applied to cells with 2.5 ml of the mixture being used per 15 cm diameter plastic dish containing 25 ml of culture medium. The following day the culture medium is removed and cells washed with 7 ml of fresh medium and replaced with 20 ml of culture medium. To generate sufficient complexity in the library this procedure is repeated 50 times to yield 1 x 10⁸ transfected cells expressing each library member.